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Isolated S sleep as a source of learning deficits induced by D sleep deprivation.

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ISOLATED S SLEEP AS A SOURCE OF LEARNING DEFICITS
INDUCED BY D SLEEP DEPRIVATION

A Dissertation Presented

By

BRUCE EDWARD RIDEOUT

Submitted to the Graduate School of the
University of Massachusetts in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 1977

Psychology

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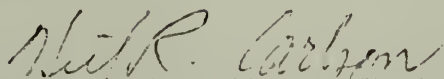
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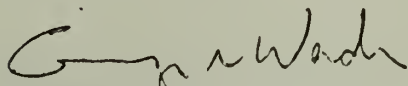
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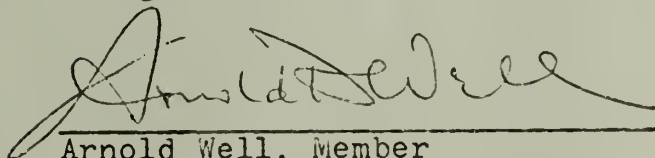
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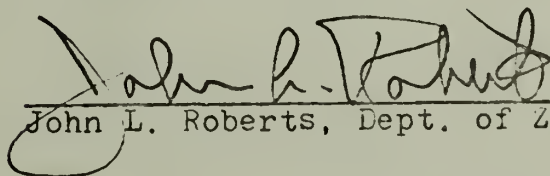
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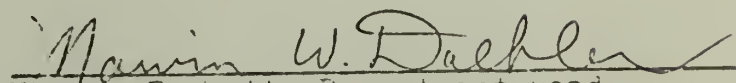
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ABSTRACT

Isolated S Sleep as a Source of Learning Deficits
Induced by D Sleep Deprivation

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Many studies have examined the results of selectively depriving organisms of desynchronized sleep (D sleep, REM sleep). These studies in general have led to the conclusion that learning deficits induced by D sleep deprivation are due to the removal of some positive influence (of D sleep) on memory consolidation. While this is perhaps the simplest conclusion, it has not been differentiated from an alternative hypothesis that such learning deficits result from periods of slow wave sleep (S sleep, synchronized sleep, non-REM sleep) that are isolated from the D sleep that normally follows. Previously, it has not been considered that S sleep may have a deleterious influence on information processing, this influence being counteracted normally by the recurrent D sleep periods. Thus, this alternative hypothesis is a refinement of the earlier D-deprivation hypothesis. This refinement must be considered if the precise origin of D-deprivation learning deficits and the functions of the sleep stages are to be understood.

The experiments described here were designed to investigate

this alternative hypothesis of an association between typical D-deprivation learning deficits and the occurrence of isolated S sleep, rather than between the deficits and the simple absence of D sleep. The standard pedestal or water-tank method of selective sleep deprivation was employed. Mice were used because they were easily classifiable as either "sleepers" (showing sleep behaviors such as hunched posture, closed eyes, slower respiration, reduced responsiveness to sensory stimulation), or "actives" (showing continuous activity) while on the D-deprivation pedestal. Ten daily trials on a complicated maze task (10 choice points) were used to assess learning. The dependent measures were time required to reach the goal box, and errors. Results were analysed using repeated measures analyses of variance. The following results were obtained:

(1) Animals showing sleep behaviors (Sleepers) while subject to brief (3 hours) post-trial D sleep deprivation, learned the maze more slowly than did animals that were continuously active (Actives).

(2) Control (no D deprivation) mice that were returned to their home cages following daily maze trials, and allowed to sleep freely, differed from the D-deprivation Sleepers but not from the Actives.

(3) If D deprivation was terminated early in the experiment, following Trial 3, Sleepers immediately (by Trial 4) caught up to Actives in performance level.

(4) Delay of the D deprivation for 3-4 hours after the daily maze trial eliminated the difference between Sleepers and Actives. Thus, a "critical period" was demonstrated for the effect.

(5) Maintaining wakefulness (by startling) in animals previously screened as Sleepers eliminated the expected D-deprivation learning deficit, while the added stress had no effect on the performance of active animals that received matched startling stimulation.

Sleepers did not differ from Actives in their initial (Day 1) exploration of the maze, or in their asymptotic performance. Both groups were also equivalent motivationally in terms of latency to enter the maze and amount of food eaten in the goal box. Running speed per se did not appear to be a major factor producing group differences; rather, the number of errors and the time required to correct an error both contributed to the D-deprivation effect, producing highly significant group differences for time to reach the goal box.

In summary, a learning deficit due to brief post-trial D sleep deprivation was found restricted to those animals showing (S) sleep. The deficit was reversible with unrestricted home-cage sleep, and also was eliminated by the delay of post-trial D deprivation for 3 hours (critical period effect). Enforced wakefulness also eliminated the learning deficit, despite the increased stress of the procedure. Thus, D-depriva-

vation learning deficits appear to be associated with isolated S sleep rather than with the simple absence of D sleep, or with the stress of the pedestal procedure.

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INTRODUCTION

Since the discovery that mammalian behavioral sleep can be differentiated into several distinct stages or states (according to electroencephalographic (EEG), electromyographic, and various other physiological criteria), a great many studies have been concerned with the mechanisms of sleep and the functions that the different stages might serve. Indeed, the problems of sleep research have been particularly intriguing because of the remarkable differences between the two most generally discriminable states of sleep. Slow wave sleep (synchronized sleep or S sleep) is characterized by reduced muscle tonus, reduced cardiac and respiratory activity, and synchronized slow wave activity in the EEG. Such features would seem to suggest energy conservation and generally anabolic function (Webb, 1974; Hartmann, 1974). In strong contrast to the features of S sleep are those of the other distinctive sleep state, desynchronized sleep (D sleep, rapid eye movement sleep, or REM sleep). This state is characterized by increased and irregular cardiac and respiratory activity, rapid eye movements, profound loss of muscle tonus, and a desynchronized (low voltage) high frequency EEG that is very similar to the EEG of wakefulness. Aside from the dramatic loss of muscle tonus, D sleep appears to be a neurophysiologically more active state, with higher oxygen consumption, generally higher neuronal firing rates, and

greater blood flow to the brain than are typically characteristic of the more "quiet" state of S sleep. It has thus been of great interest to consider what role this active, relatively non-conserving sleep state might be fulfilling for an organism that is otherwise not interacting with its environment in any apparently profitable way.

A natural approach to investigating the function of D sleep is to deprive the organism of this state. Since S sleep (SS) and D sleep (DS) normally occur in a cyclic fashion, with wakefulness typically leading to a period of SS before the occurrence of DS, DS deprivation (DSD) can be accomplished by awakening the sleeping subject when the first signs of DS appear. When the subject returns to sleep SS occurs for a time before the signs of DS once again appear. Although the latency of onset of signs of DS may become greatly reduced as this awakening procedure is repeated (to the point of almost immediate onset following the wakefulness-sleep transition), in the short run an essentially selective deprivation of DS does occur, which is concurrent with large amounts of SS. This method of DSD led to the discovery of the now well established "rebound" phenomenon. Selective deprivation of DS is followed by a higher than normal proportion of DS during subsequent unrestricted "recovery" sleep. This phenomenon has led to the suggestion of a "biological need" for DS.

It should be added that in addition to the difficulty of

depriving of SS without depriving of DS as well (due to the normal sequence of states, mentioned above), comparatively little research effort has been devoted to the question of S sleep need. Nonetheless, research that has been attempted still points to some priority for DS under circumstances of deprivation. Agnew, Webb, and Williams (1967) compared DSD with deprivation of stage 4 (the deepest non-REM stage, with the highest proportion of slow waves) in humans. Stage 4 rebound did occur, but it was less pronounced than the DS rebound seen in the DSD condition. In addition, even in the stage 4 deprivation condition, stage 4 rebound was followed by DS rebound on subsequent nights. In another study Levitt (1967) found that DSD led to DS rebound, and that total sleep deprivation also led to a higher than normal proportion of DS (in rats). He therefore suggested that "at higher levels of sleep need" DS need "has the highest priority."

The method of DSD by monitoring and awakening is of course very difficult with large numbers of animal subjects, and this difficulty has led to the great popularity of a second, more practical method of DSD. At an early point in sleep research Jouvett, Vimont, Delorme, and Jouvett (1964) suggested the use of the pedestal (or flower pot, or water tank) technique as a practical method of DSD in animal subjects. With this method the animal is placed on a small pedestal surrounded by water. While so situated the animal can enter SS with

its moderate muscular and postural relaxation; DS, however, involves such a drastic loss of muscle tonus that onset of this state causes the animal to droop its head into the water and awaken, to lose its balance, or (as is most typical after a little practice) to awaken abruptly as the muscular relaxation of DS commences. This method was originally employed with cats, but has now been extensively used with rats and mice as well. The equivalence of the pedestal and awakening procedures in the rat has been demonstrated in terms of the amount of DS occurring, and also in terms of the extent of rebound shown during recovery sleep (Morden, Mitchell, and Dement, 1967).

Despite its practicality, the pedestal method of DSD is somewhat problematical due to the stressful nature of this procedure (Stern, 1969; Mark, Heiner, Mandel, and Godin, 1969; Stern, Miller, Cox, and Maickel, 1971). In the great many studies of the behavioral and biochemical effects of pedestal DSD, researchers have dealt with the problem of stress as a confounding factor in a number of ways. For instance, Morden et al. (1967) suggested that animals placed on a larger pedestal could serve as appropriate controls in terms of both their sleep patterns (presence of DS) and their exposure to the water tank environment. Other studies, however, have reported that the large platform control procedure causes some limitation of DS to around 50-75% of baseline values (Duncan, Henry, Karadzic, Mitchell, Pivik, Cohen,

and Dement, 1968; Mark et al., 1969; and Mendelson, Guthrie, Frederick, and Wyatt, 1973). Thus, the results of studies using this control procedure have been placed in question. An alternative stress control devised by Stern (1971) involves immersion of the animal in 19° C water for one hour per day and the normal home cage environment for the remainder of the time. Stern reports that this treatment causes adrenal hypertrophy (a measure of stress) equivalent to that induced by pedestal DSD (in the rat). Pearlman (1971) also used this stress control but emphasized that this procedure was more stressful than the pedestal treatment, with control animals (rats) becoming exhausted and frequently requiring removal from the water to avoid drowning.

More recently Pearlman has dealt with the confounded stress effect by substantially reducing the time spent on the DSD pedestal, thereby reducing the total amount of stress as well. Previously, experimental animals were typically subjected to prolonged DSD on the pedestal. Although prolonged DSD (3-5 days) may still be of value in biochemically oriented studies, the use of brief (<6 hours) DSD has provided a definite advance in efforts to clearly demonstrate the involvement of DS in learning. In a number of studies Pearlman has shown that in rats about 3 hours of DSD immediately following a training session causes a learning deficit in such tasks as brightness discrimination (Pearlman and

Becker, 1973), shuttlebox avoidance (Pearlman and Greenberg, 1973), latent extinction (Pearlman, 1973), bar-press (Pearlman and Becker, 1974a), and serial reversal (Pearlman and Becker, 1974b). In these experiments control subjects received the same DSD treatment, but with the onset of DSD delayed for two hours. Thus the deficits in task acquisition could not be due to the stress of the pedestal procedure unless temporal proximity of the stress to the training session is of key importance. However, Pearlman also accomplished brief post-trial DSD (immediate vs. delayed) by means of low dosage drug treatments, using imipramine and chlordiazepoxide on alternate days to abolish DS for short periods (Pearlman and Becker, 1974a, 1974b). In these cases, therefore, the stress of the pedestal was completely avoided, and the time spent in DS per 24 hours did not differ significantly among the three groups having immediate DSD, delayed DSD, or no DSD (in home cage). A parallel development that also demonstrated a connection between learning and DS was the finding that an enhancement of DS typically followed a training session and was associated with improved performance in subsequent sessions (Lucero, 1970; Leconte and Hennevin, 1971; Smith, Kitahama, Valatx, and Jouvét, 1972; Leconte, Hennevin, and Bloch, 1974; Fishbein, Kastaniotis, and Chattman, 1974; and Smith, Kitahama, Valatx, and Jouvét, 1974). (Both rats and mice were included in these findings.) This augmentation of DS was found

to be due to an increase in number rather than duration of DS phases (Leconte et al., 1974; Smith et al., 1974).

Fishbein (1970, 1971) employed mice in DSD experiments and found that the pedestal procedure (prolonged) could cause deficits in learning if it were used either before or after a training session. In addition, Fishbein, McGaugh, and Swarz (1971) have demonstrated in a different manner that DS is involved with memory consolidation. By giving 2 days of DSD immediately following one trial of passive avoidance training, and then administering electroconvulsive shock at various times subsequent to the end of DSD, these authors showed that DSD served to prolong the labile phase of memories concerned with the training experience. After 2 days of DSD mice were still susceptible to the amnesic properties of the ECS. The amnesic effect was apparent at DSD-ECS intervals up to 1 hour (3, 6, or 12-hour delays of ECS following DSD prevented the amnesic effect). To some extent Fishbein's use of mice, even in prolonged pedestal treatment (3-7 days), serves to lessen the importance of stress as a possibly confounded factor. In their reaction to the pedestal mice appear to differ strikingly from rats. Given the opportunity, mice will remain active and climb about on the underside of the cage top; rats will not. Although systematic measures of physiological stress indicators (such as adrenal hypertrophy) have not been taken, Fishbein et al. (1971) point out that

as much as 5-7 days of DSD on the pedestal do not produce noticeable changes in open field behavior. This contrasts with the general transient post-DSD hyperactivity that has been reported in rats (Pearlman, 1971), which may be related to the immobility that rats are subject to while on the pedestal.

Although many studies have yielded evidence for the involvement of D sleep in memory consolidation, the nature and to some degree the existence of this involvement remains controversial. Doubts have been sustained in part by reports of negative findings such as those of Miller, Drew, and Schwartz (1971) and Albert, Cicala, and Seigal (1970). The importance of stress in producing whatever effects result from the pedestal method of DSD also remains a central issue (see, for example, Pearlman, 1976). The question also remains as to why DSD prior to training does not enhance learning rather than retard it by means of the rebound of DS that follows the DSD. Presumably a biochemical imbalance may be mediating this effect, and some studies measuring levels of putative central neurotransmitters during and following DSD have in fact given some evidence in support of this basic idea (Pujol, Mouret, Jouvet, and Glowinski, 1968; Schildkraut and Hartmann, 1972; Cramer, Tagliamonte, Tagliamonte, Perez-Cruet, and Gessa, 1973; and Kovacevic and Radulovacki, 1976). It seems likely that the effect of DS is not due only to the amount of time spent in this state, but also to the density of DS

phenomena and rates of neurotransmitter synthesis and utilization. Since our ideas on the function of DS are largely those that can be inferred from the effects of DSD, any other variable that is altered uncontrollably during DSD should be investigated for its influence on DSD effects. This point is particularly germane in approaching the hypothesis to be examined in the study to be described here.

The experiments described below were designed to test the hypothesis that learning deficits associated with DSD are due to the occurrence of isolated periods of S sleep. Previous studies that found DSD-induced learning deficits led to the conclusion that the deficits resulted from the loss of some positive influence of DS on memory consolidation. While this is perhaps the simplest conclusion, in the previous research designs this conclusion could not be differentiated from the alternative hypothesis that will be considered here; that the DSD learning deficits are the result of isolated SS, which itself has a deleterious influence on information processing, this influence normally being counteracted by the cyclically occurring DS. This hypothesis is thus a more complicated interpretive refinement of previous conclusions, but nonetheless one that must be considered if the precise origin of DSD learning deficits and the functions of the sleep stages are to be understood. As mentioned above, the function(s) and effects of SS have received relatively little attention. The possibility of a negative influence of SS on information

processing has not been dealt with at all. We may note again that repeated cycles of sleep stages within a sleep period are virtually universal among the many species thus far investigated in sleep studies, and in the first cycle DS generally follows SS. These features are quite consistent with the hypothesized relation between SS and DS. Indeed, from a neurophysiological viewpoint Ephron and Carrington (1966) considered DS as the expression of a homeostatic mechanism that functions in opposition to SS to maintain appropriate levels of "cortical tonus."

Although investigation of the hypothesized effect of isolated SS would most directly involve a careful monitoring of EEG in individual animals during DSD, a grosser and more practical method is available if mice are used as the experimental subjects. As mentioned earlier, if mice have the opportunity to climb while being subject to DSD by means of the pedestal method, they will do so and thereby remain active at least part of the time. In a preliminary investigation it was found that 40-50% of randomly selected mice of a hybrid strain showed some sleep posturing and decreased responsiveness to stimulation during brief (4-5 hours) pedestal DSD, while the remaining animals were generally active, climbing on the underside of the cage top, grooming, etc. In the current study, therefore, DSD was accomplished by the typical pedestal procedure, with animals monitored behaviorally and classified according to responsiveness. Brief DSD followed

daily sessions of training on a maze task. As a result of the animals' self-classification, the groups of animals considered as "sleepers" and "actives" were not composed according to a truly random assignment. Various control procedures were therefore employed to ascertain that any effects supposedly due to isolated SS were not actually the result of other confounded variables.

EXPERIMENT ONE

The purpose of Experiment 1 was to determine whether mice showing sleep behaviors during DSD learned a maze task more slowly than mice given the same DSD treatment but showing no sleep behaviors.

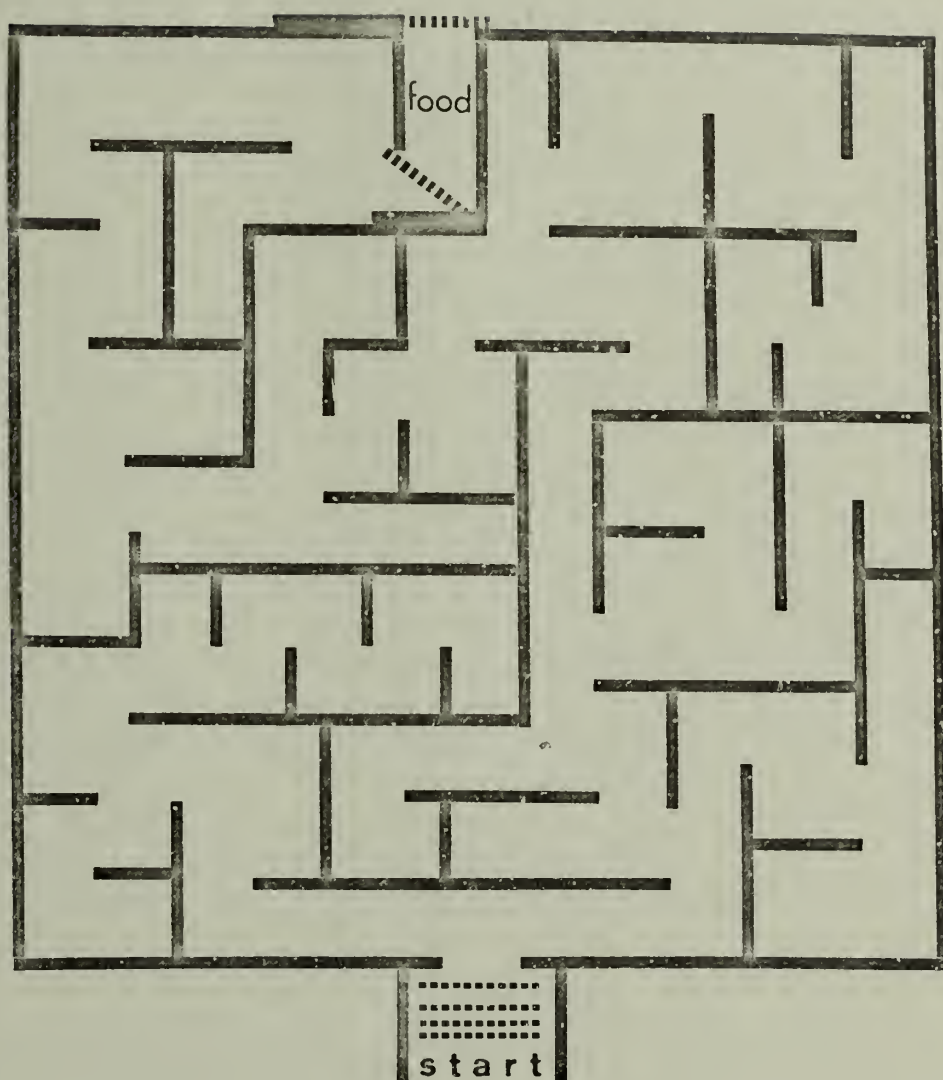
Method

Subjects. The subjects for Experiment 1 were 25 male hybrid mice, strain B6D2F₁/J, obtained from Jackson Laboratory, Bar Harbor, Maine. All animals were between 4 and 6 weeks of age during the period of study, and each weighed between 15 and 20 grams.

Apparatus. A maze was used to assess learning. The maze was constructed of Masonite and covered with two coats of polyurethane. The plan of the maze is shown in Figure 1. Passageways were approximately 5 cm. in width and depth, and the total runway length to the food chamber (without errors) was approximately 178 cm. During training sessions the top of the maze was covered with a sheet of transparent plexiglass (1/8 inch thickness). An animal entered the maze by means of a Masonite ramp, 18 cm. X 28 cm., which was inserted into its home cage and led up to the entrance of the maze, which was thus elevated above the level of the home cage.

Deprivation of DS was accomplished on small plexiglass pedestals (3 cm. diameter) designed using dimensions taken from Fishbein (1970). The pedestals were placed in transparent

Figure 1. Floor plan of maze used to asses learning.



plastic cages identical to home cages (18 cm. X 28 cm. X 13 cm. deep), but without bedding material. The pedestal cages were filled with water up to the level of the pedestal, but not over the pedestal top. The cage tops on all home and pedestal cages were identical and enabled an animal inside to climb and (in the pedestal cage) to return to the pedestal easily (without getting wet). The water was shallow enough to allow an animal's hind legs to touch the floor of the cage while the animal was in the water. The water temperature varied from 30° -21° C, as it cooled to room temperature (21° C).

Procedure. In this and the subsequent experiments animals were fed daily rations of wet food mash made by mixing 2 parts powdered rat food¹ to 3 parts water by volume. A ration of approximately 7.4 cc. (2 teaspoons) of mash was sufficient to maintain a mouse at 90-95% of its free-feeding body weight, while still promoting reliable motivation at the daily training (and feeding) hour. In order to reduce the effect of initial stress of the pedestal procedure on maze learning, and to allow DSD behavior to stabilize before exposure to the maze, all animals initially had 3 daily 3-hour periods of DSD. These 3 days with pedestal training were followed by one day without DSD, and then by 10 daily sessions of training on the maze. Concurrent with pedestal training each animal was exposed to a maze ramp for 10

¹Purina Powdered Laboratory Chow

minutes daily (for 3 days), or until a small amount of food was eaten at the top of the ramp. Only an occasional animal did not eat within the allotted 10 minutes, and this failure to eat never occurred after the first day of ramp training. The animals were also allowed to explore an adjoining cage top to which the ramp led. This ramp training accustomed the animals to finding food at the top of the ramp and having the opportunity to explore. The effect of this procedure was to expedite entrance into the maze during the maze training that followed. The sequence of preliminary training procedures was also preparatory for the subsequent maze training procedure in that the ramp led to a small amount of food (and exploration), which was followed by feeding on the daily ration of food in the home cage for 30-45 minutes, which was then followed by 3 hours of pedestal DSD. All experimental procedures began within the first 2 hours of light in a 12-hour light, 12-hour dark schedule, in order to facilitate sleep during DSD.

During training on the maze task 3 wet food pellets (Noyes, 20 mg.) were placed in the goal chamber. Each animal had one trial per day for 10 days. During a trial the ramp was inserted into the animal's cage, the animal climbed up the ramp, explored the maze, found the food and ate at least 2 of the 3 pellets. When the animal entered the goal box a door was closed behind it, preventing the animal from returning to the other parts of the maze. After eating at

least 2 of the pellets, the animal was allowed to exit from the maze into a small cardboard box which was removed to the animal's home cage, where the animal climbed out. Within 15 minutes the animal received its daily ration of food mash. The animal was placed on its pedestal for DSD after being in its home cage for 50 minutes, or immediately if it showed pre-sleep behaviors such as nest building and sleep posturing. In general, pre-sleep behaviors occurred after 30-45 minutes of feeding. The maze was washed daily after all training sessions were completed, and water in the pedestal cages was also changed on a daily basis.

All animals had DSD for 3 hours, DSD thus terminating $3\frac{1}{2}$ -4 hours after the maze trial. Animals were scored on their behavior every 10 minutes during DSD. If they were not obviously active (and scored accordingly), they were tested in their responsiveness to a visual stimulus (cardboard flag, 10 cm. X 10 cm.) that was waved close to the side of the cage and also above the cage. This was not simply a test for eye closure because due to lighting conditions the stimulus object also created a shadow to which animals with closed eyes would often respond. In this latter case the response was similar to the typical wakeful response, with orienting, stretching toward the stimulus, and sniffing. Use of the stimulus above the cage top

immediately over the animal appeared to have an auditory component as well, and this component sometimes seemed stronger than any other aspect of the stimulation: occasionally animals that were not responsive to lighting interruption (shadow of stimulus) would move their ears, arouse, and orient upward toward the stimulus. This effect was probably due to some slight change in background noise for the animal. Animals that did not respond always had the same posture. They were hunched over, with eyes closed and generally slower respiration; they also showed nodding that typically led to brief postural readjustment. Occasionally, posture would droop or balance would be lost to the point of an animal's falling into the water, but this was not typical of most animals.

Although animals were generally consistent in their pedestal behavior throughout the 10 days of maze training, final classification of animals as "sleepers" or "actives" was not made until the end of the experiment. Classification was based on the average number of "sleep" scores per day during DSD. A "sleep" score was defined as the animal being non-responsive to the stimulation procedure, or responding only partially while in the typical non-responsive posture. As a control for consistency across the 10 days, to prevent single days with many sleep scores from inflating the average, a maximum of 5 sleep scores per day was used in calculating the average. An animal was considered a Sleeper

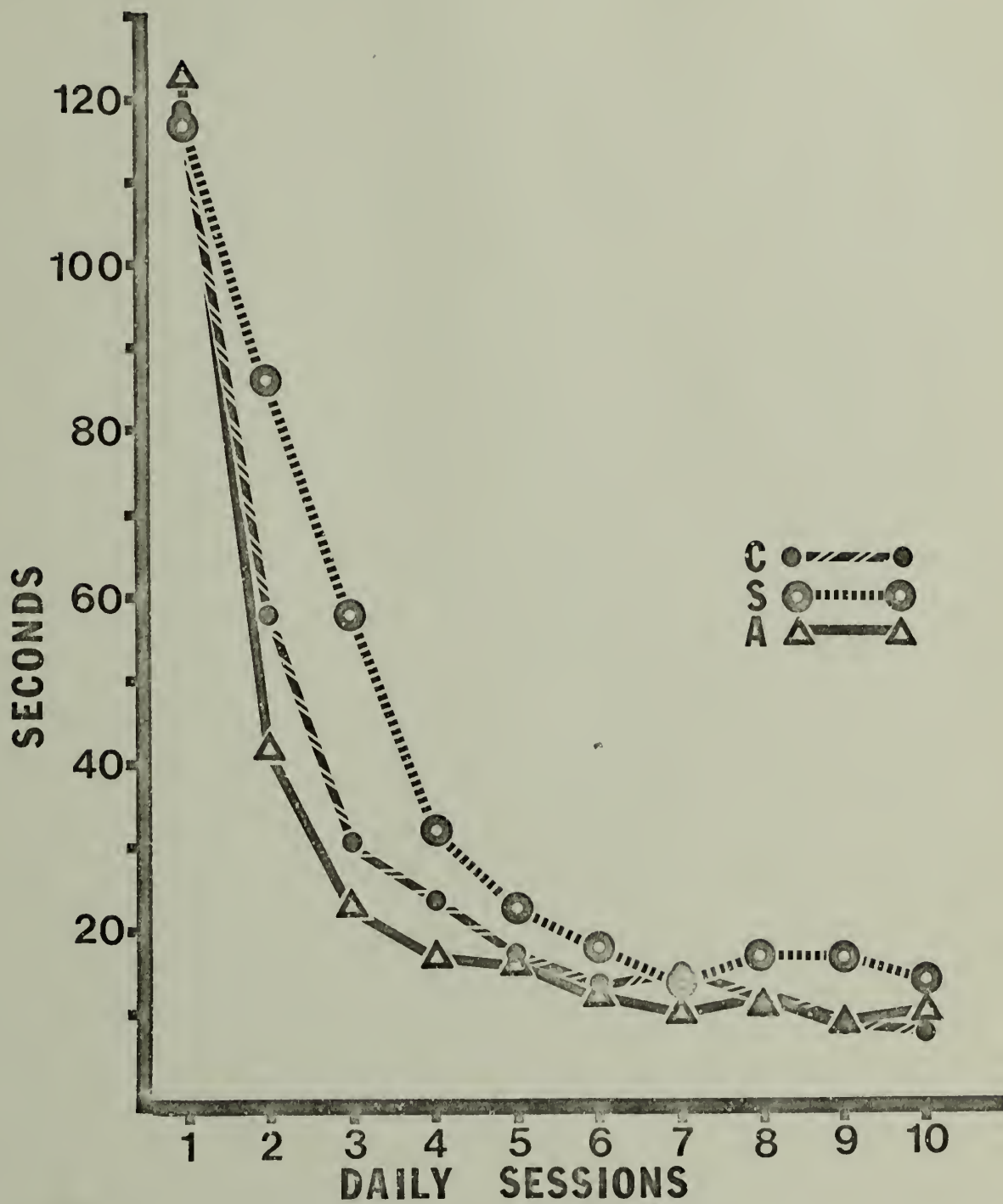
when the mean number of sleep scores was at least 3. Animals with sleep scores of less than 2 were classified as Actives. Animals with intermediate mean scores were considered ambiguous for the purposes of this experiment and were not used in the data analyses.

Two dependent measures were used to assess learning of the maze task: time to reach the goal chamber and number of errors. The time measurement began when the animal entered the maze, and an error was defined as a wrong turn at any of the choice points in the maze. A wrong turn was considered as a single error regardless of what the animal did subsequent to the wrong turn but prior to returning to the choice point. A reversal of direction (while on the correct path to the food) was considered as a single error, but subsequent turns from the main path were counted as additional errors.

Results

After classifying the animals by the procedure described above, the groups of Sleepers and Actives consisted of 8 and 11 animals, respectively. Figure 2 summarizes the results of Experiment 1 in terms of time to reach the food chamber. As expected, on Day 1 the two groups did not differ by much, and a t test showed no statistically significant difference ($t_{17} = -.177$). Thus the two groups did not appear to differ initially in their success at exploring the maze

Figure 2. Summary of Experiments 1 and 2. Curves show performance of Sleepers (S), Actives (A), and normal controls (C).



and finding the food. Subsequent to Day 1, however, the groups differentiate and the Sleepers show slower acquisition of the task. A repeated measures analysis of variance on the data from Days 2-10 indicated that the groups differed ($F_{1,17} = 14.76$, $P < .001$). The effect of sessions (days) was also significant ($F_{8,136} = 16.92$, $P < .001$) as was the groups X sessions interaction ($F_{8,136} = 2.98$, $P < .004$). It should be noted here that these group differences are not the result of Sleepers being generally more sluggish when exposed to the maze. The animals in the two groups were equally alert during training and showed no difference in latency to climb the ramp and enter the maze. Virtually all animals climbed the ramp immediately on Days 2-10, and latency never exceeded 5 seconds. An analysis of variance on the errors of the two groups showed results similar to, but not as striking as the results of the time data analysis. The group differences approached, but did not reach significance ($F_{1,17} = 4.09$, $P < .059$). The effect of sessions was significant ($F_{8,136} = 13.29$, $P < .001$) as was the groups X sessions interaction ($F_{8,136} = 2.22$, $P < .029$). Although the group differences in the error data did not reach the level of significance seen in the time data, interpretation of the error data is not simple, and the results of the two measures are not necessarily contradictory. In this particular maze all errors are not equivalent in terms of time-loss for the animal. For this reason errors are not quanti-

liable in the simple fashion that the counting of wrong turns and direction reversals would suggest. Thus the measurement of errors in this case is complicated, and the method used here was perhaps inappropriate, at best not interpretable in a straightforward manner. In the subsequent experiments described below, analysis is limited to measurement of time to reach the food chamber.

The less striking results in the error data may seem to indicate that some of the group differences could be due to slower running speed for the Sleepers, but this did not appear to be the case. Rather, the Sleepers appeared to take longer correcting their errors, and to be slightly slower in making decisions at choice points. That the Sleepers were not simply slower runners is also indicated by asymptotic performance of the two groups, which was essentially equivalent, particularly when compared to the pronounced group differences found earlier in maze training.

Closer examination of the data indicated that early in maze training (when most of the group differences were appearing), time scores of Sleepers exhibited substantially larger variance ($\times 4$) than did those of the Actives. As this difference could indicate an underestimation of α in the analysis described above, the analysis was repeated using logarithms of time scores. The results of this analysis were generally consistent with those of the previous analysis, with significant main effects for groups ($F_{1,17} = 11.21$, $P < .004$) and

for sessions ($F_{8,136} = 31.15$, $P < .001$), but with the previously significant interaction (groups X sessions) becoming non-significant.

In summary, Experiment 1 did indicate that the effect of the pedestal method of DSD on the learning of a maze task varies depending on the behaviors of the subjects during DSD. Animals showing consistent sleep behaviors during the brief DSD periods that followed daily trials in the maze also showed slower learning of the maze in terms of time to reach the goal chamber when compared to animals that were active throughout DSD. Subsequent experiments were designed to further elucidate these differences.

EXPERIMENT TWO

The results of Experiment 1 are interesting, but they require further examination in several ways. Perhaps most immediate among these is placing the effect at issue into proper perspective against the performance of normal (no DSD) animals on this task. Is the differential performance shown by Sleepers and Actives small in comparison to the difference between all DSD and normal animals? Or are Actives showing learning that is essentially equivalent to that of normals? Experiment 2 was designed to answer these questions by testing normals on the maze task used in Experiment 1.

Method

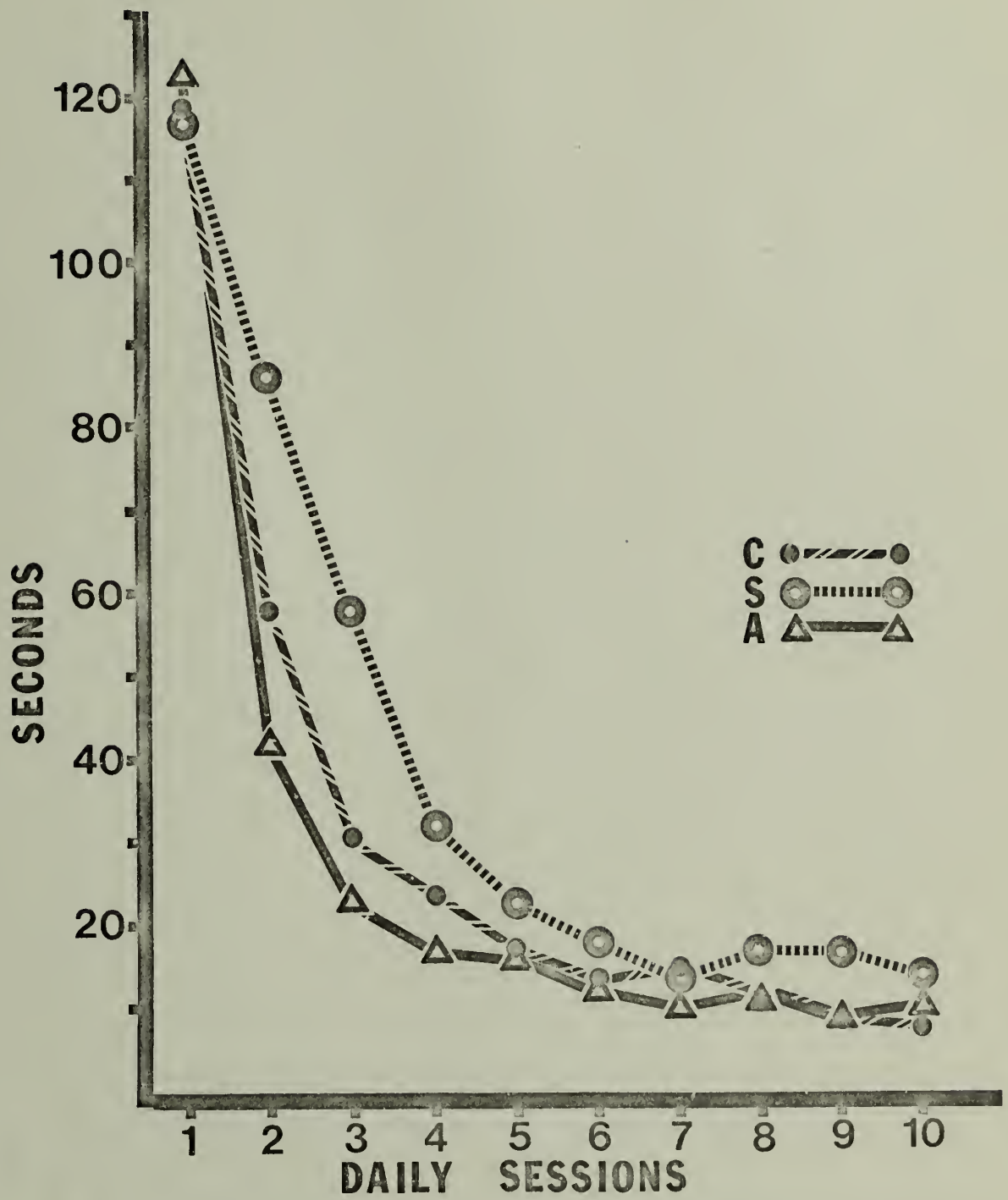
Subjects. The subjects for this experiment were 21 mice of the same type used in Experiment 1.

Procedure. All animals were maintained and trained in the same ways as described for Experiment 1. In this case, however, animals were not given DSD following maze training and feeding. Instead, all the animals remained in their home cages and were allowed to sleep freely. Latency in showing curled-up sleeping posture was noted.

Results

The results of Experiment 2 are summarized in Figure 2 in

Figure 2. Summary of Experiments 1 and 2. Curves show performance of Sleepers (S), Actives (A), and normal controls (C).



terms of the performance of the entire group of normal animals. Two of the original 21 animals showed unusually long latencies in maze entry (15 seconds), and so were excluded from the analysis. It can be seen that the group data here are quite similar to those of the Actives in Experiment 1, but the normals showed slightly higher mean scores for Day 2 and Day 3. However, a repeated measures analysis of variance indicated no difference between the normals and the Actives from Experiment 1. On the other hand, the normals were found to differ significantly from the Sleepers of Experiment 1 ($F_{1,25} = 11.22$, $P < .003$), and no significant interaction occurred. Variability in the data from the normals was generally consistent with that shown by the Actives, and only slightly higher on Days 2-3 (variance about X 1.5). Thus a sizeable difference existed between the variability seen in the normals and that found in the Sleepers. Repeating the analyses using the logarithms of the scores had no effect on the interpretation of the results, however, for the group differences remained non-significant between Actives and normals, and showed a slight increase in level of significance for the difference between Sleepers and normals ($F_{1,25} = 14.47$, $P < .001$). In all the analyses the effect of sessions was significant at $P < .001$.

Although there can be no certainty as to which of the normal animals might have been classifiable as DSD Sleepers in a different experimental design, it was of interest to

see if behavior on the pedestal during the third day of pedestal training (prior to maze training) could serve to predict in any way either maze performance or home cage behavior subsequent to maze training sessions. That is, were those animals that were more likely to be classified as DSD Sleepers slower in their learning of the maze? In addition, were these animals more likely to sleep or show a shorter latency to sleep in their home cages following a training session? To investigate these questions 8 animals that were generally less active and showed sleep behaviors while on pedestals were compared with the 11 remaining normals that were generally active. An analysis of variance indicated that these two subgroups of the 19 normals did not differ significantly in their learning of the maze. No differences in behavior while in the home cage were seen. Animals in both groups varied in their sleep latency from 45-90 minutes over the 10 days of training on the maze, and all animals slept within the $3\frac{1}{2}$ -3 $\frac{3}{4}$ hours following maze training that corresponded to the feeding and DSD period in Experiment 1.

In summary, Experiment 2 demonstrated that the maze performance shown by active DSD animals in Experiment 1 was essentially equivalent to that of normal mice that had no DSD while learning the maze. The group of animals classified as Sleepers in Experiment 1, however, remained statistically differentiable from the normals of Experiment 2.

In addition, there was no suggestion that the 8 less active of the normal mice, those of the normals with the highest potential for being DSD Sleepers, were any slower in learning the maze than the more active subgroup of the normals, or were any different in their home cage behavior following learning sessions. These results lend further support to the hypothesis at issue in this study, that learning impairment associated with DSD is restricted to animals showing (S) sleep behaviors during this period of deprivation, since the active animals are also subject to simple removal of D sleep and they show no learning deficit when compared to normals.

EXPERIMENT THREE

Experiment 3 was designed to further examine the maze performance of Sleepers and Actives in comparison to normals, in this case by early termination of the post-trial DSD procedure, keeping all animals in their home cages after the third and subsequent training sessions. Thus, DSD occurred after maze training (and feeding) only on Days 1 and 2.

Method

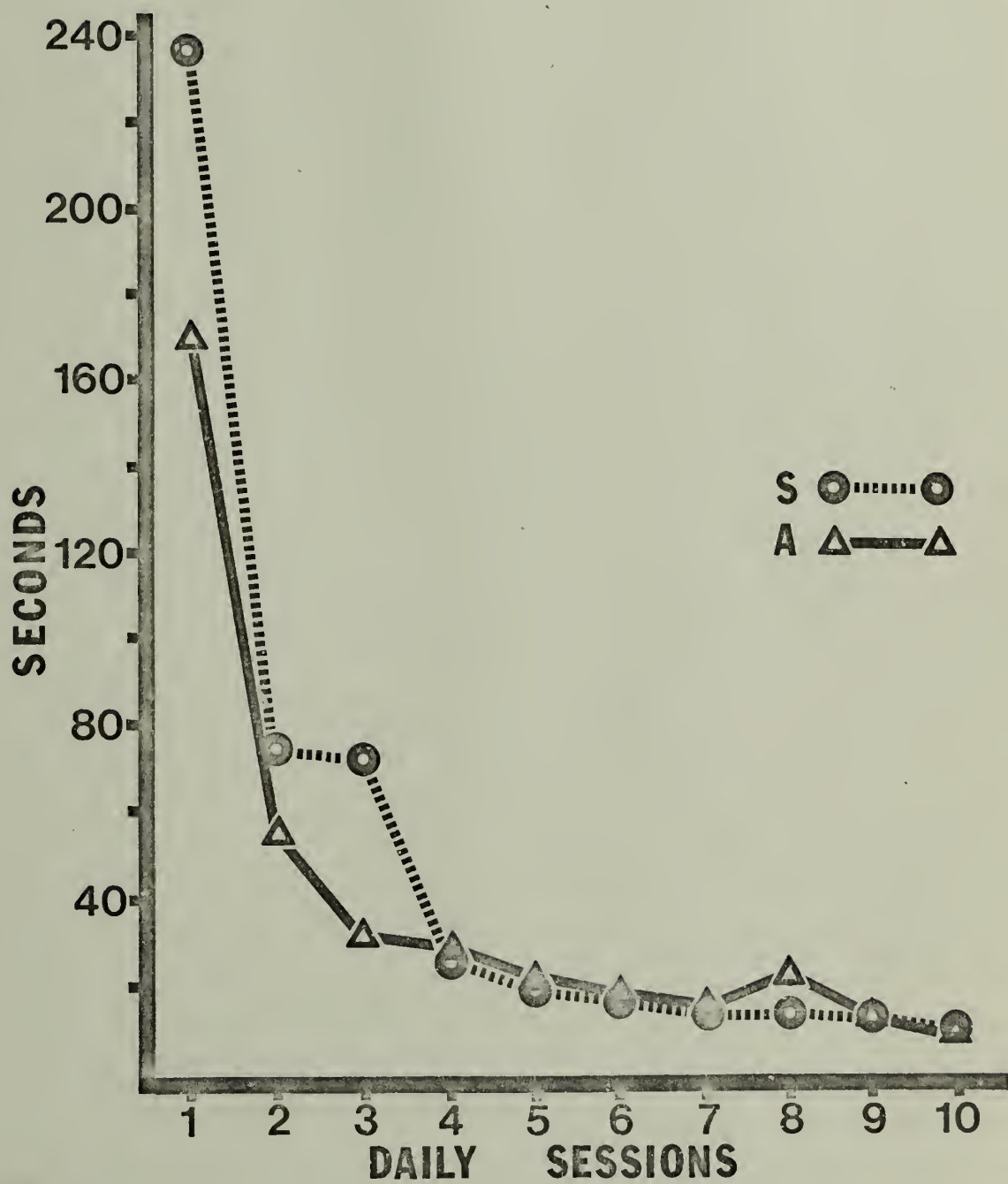
Subjects. The subjects for Experiment 3 were 24 mice of the same type used in the previous experiments.

Procedure. Procedures were essentially the same as those used in Experiment 1, with all mice familiarized with ramps and DSD pedestals prior to any training on the maze. During maze training, however, the 3-hour DSD periods were administered only on Days 1 and 2. As a result, classification of animals as Sleepers or Actives was based on 2 periods of DSD behavior rather than 9, as was the case in Experiment 1. Procedures starting on Day 3 were the same as those in Experiment 2.

Results

The performance of the two groups is shown in Figure 3. Six of the original 24 animals were not used in the analysis

Figure 3. Summary of Experiment 3. DSD administered only on Days 1 and 2. Curves show performance of Sleepers (S) and Actives (A).



because of long latency to enter the maze ($n=3$) and failure to eat at the goal box ($n=3$), which suggested they were not motivationally equivalent to the remaining 18 animals. Among these 18 animals 10 were classified as Sleepers. Although the time to reach the food on Day 1 was substantially longer for the Sleepers, and longer for both groups than had been the case in Experiments 1 and 2, variability in the data from both groups was high, so the groups did not differ significantly ($t_{16} = 1.380$). On Days 2 and 3 performance of Sleepers and Actives was quite similar to that of the corresponding groups from Experiment 1. An analysis of variance on the data from these two days indicated that the groups did differ significantly ($F_{1,16} = 4.90$, $P < .042$), while the effect of sessions and the groups X sessions interaction were non-significant. As previously encountered in Experiment 1, variability among Sleepers was substantially larger than among Actives. The mean variance for the Sleepers over the two days was approximately 5 times that of the Actives. The analysis was therefore repeated using logarithms of scores, and this second analysis showed that the main effect of grouping was still significant ($F_{1,16} = 9.08$, $P < .008$). The effect of sessions this time approached significance ($F_{1,16} = 4.38$, $P < .053$), but the groups X sessions interaction remained non-significant. As can be seen in Figure 3, the two groups were virtually equivalent in performance after Day 3, the day following the last post-training

DSD. Additional analyses also indicated that the Sleepers and Actives of Experiment 3 were not different from their corresponding groups in Experiment 1 (on Days 2 and 3).

Thus, Experiment 3 shows again in general that classification of some animals as Sleepers is not equivalent to selecting a group that is basically less bright. Under home cage conditions, Sleepers learned the maze task at a normal rate. Indeed, after DSD was stopped the Sleepers did not show a learning curve parallel to that of the Actives, but instead required only one day with unrestricted sleep following the training session in order to catch up to the Actives in performance. This rapid recovery is of interest and its implications will be discussed later. It is also interesting to note that termination of DSD does not produce in the learning curve of the active animals any obvious discontinuity similar to that seen in the learning of the Sleepers. This presents evidence again that the performance of Actives is not affected by the DSD procedure, but involves an essentially normal level of performance. Finally, it should be pointed out also that the large difference in within-group variability for the two groups, which has been associated previously with the DSD procedure, was completely eliminated with the discontinuation of the DSD, and this was primarily due to a decrease in variance for the Sleepers. This difference continued for an additional day in Experiment 1.

EXPERIMENT FOUR

Experiment 4 was designed to test whether the effect of DSD on Sleepers was dependent upon the occurrence of the DSD within a "critical period" following the training session. The basic procedure used was similar to the delayed DSD method used as a control condition by Pearlman and Becker (1973). Therefore, the question at issue was: Can a delay of the DSD procedure for three additional hours eliminate the learning difference shown between Sleepers and Actives in much the same way that DSD learning deficits found by other researchers seemed to be eliminated by the delaying of DSD? Under delayed DSD conditions, an absence of Sleeper-Active differences would support an association between the DSD deficits found in the present study and those observed previously.

Method

Subjects. The subjects were 22 mice of the same type used in the earlier experiments.

Procedure. As in the previous experiments, all animals were initially exposed to the experimental environment. Training on the maze was accomplished as usual, as was feeding. At the time when the animals would have been placed on their pedestals (in a post-feeding DSD procedure), they were allowed to sleep freely in their home cages and any remaining

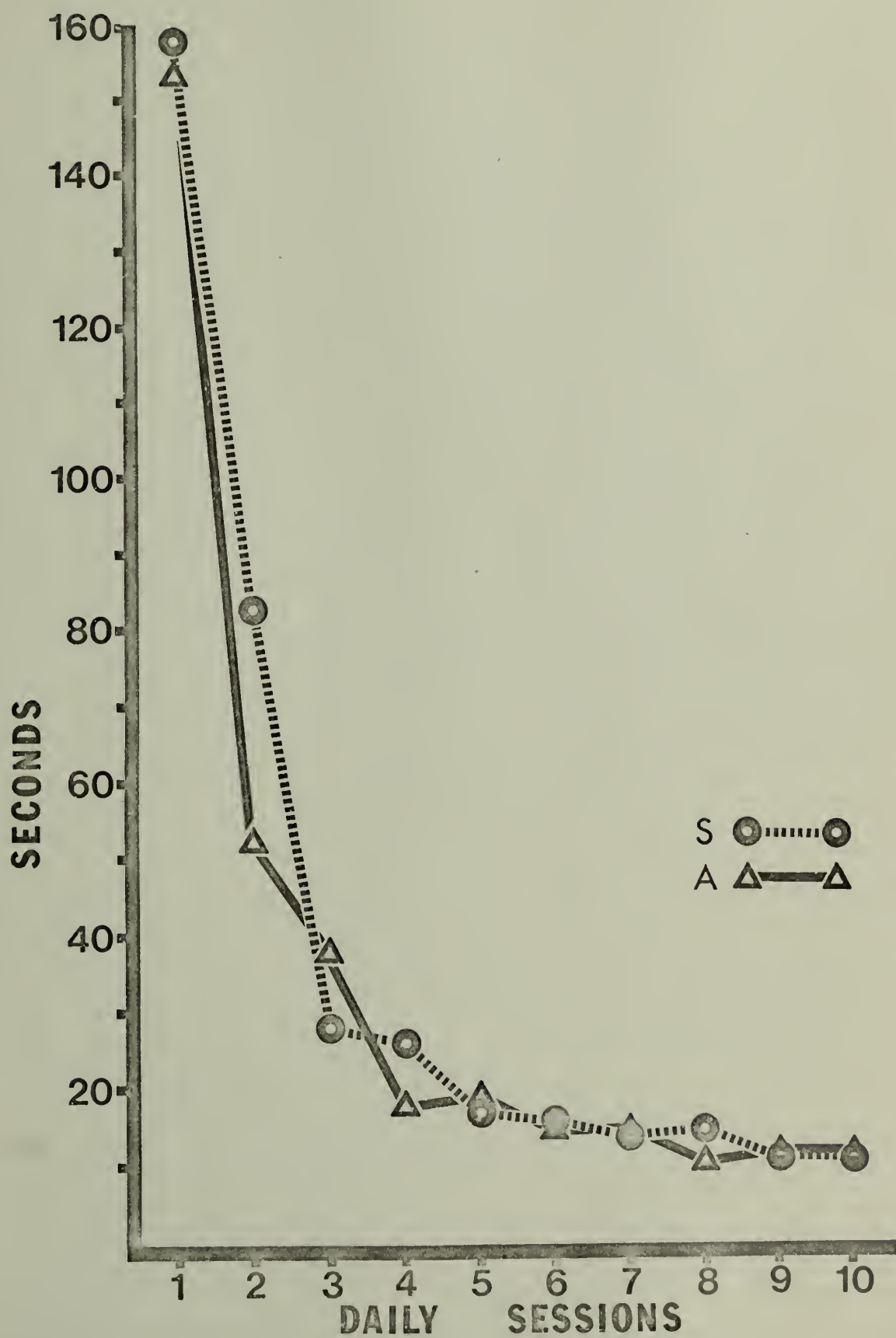
food was removed. Food was returned in $2\frac{1}{2}$ hours. The animals were placed on DSD pedestals after 45 minutes of feeding, or immediately if they showed pre-sleep behaviors. Removal of food, followed later by a repeat of the feeding procedure, was used in order to approximate closely the pre-DSD procedure used in Experiment 1.

Results

Figure 4 shows the performance of Sleepers and Actives resulting from the delayed DSD design. Two animals were not considered in the analysis due to their long latencies in maze entry. Although the amount of sleep shown by the mice was somewhat less than in previous designs, application of the criteria used earlier yielded 8 animals classified as Sleepers, 12 as Actives, and no ambiguous subjects as defined earlier.

As can be seen from the performance curves, the groups were essentially equivalent on Day 1 (t test non-significant). The mean score for the Sleepers on Day 2 (83 sec.) was elevated above that of the Actives (52 sec.), but this was due to a single high score of 323 sec., without which the mean for Sleepers was 49 sec. Analyses of variance showed no significant differences between the two groups, and neither of the groups differed from the normals of Experiment 2. As in most of the previous experiments, all analyses showed that the effect of sessions was significant at $P < .001$. With the

Figure 4. Summary of Experiment 4. DSD administered after a delay of 3-4 hours. Curves show performance of Sleepers (S) and Actives (A).



exception of elevated variance within the Sleepers for Day 2 (due to the single aberrant score), there was no occurrence of the increased variance previously associated with the DSD learning deficit in Experiments 1 and 3.

Aside from demonstrating again that except for their response to the pedestal situation Sleepers do not differ from Actives in their learning ability, these results further clarify the effects under study. By supporting the hypothesis that the effect in question has a critical period, these results also suggest a correspondence between the present DSD-induced learning deficit found in (and restricted to) Sleepers, and learning deficits demonstrated for other tasks in previous research. We are therefore more firmly led to the view that the findings of this study may be generalized to other DSD research, and that they are not simply due to some special feature of the maze task.

EXPERIMENT FIVE

If Sleepers and Actives learn the maze at essentially equivalent rates under normal circumstances, and differ in the DSD situation only as a result of isolated S sleep in the Sleepers, then keeping the Sleepers awake should eliminate the DSD learning differential. Wakefulness of the Sleepers in this experiment was enforced by means of striking the cage tops when sleep posturing occurred. In order to control for the additional stressful effect of this procedure, each Sleeper was matched to an Active which received equivalent stimulation.

Method

Subjects. The subjects for Experiment 5 were 22 mice of the type described earlier.

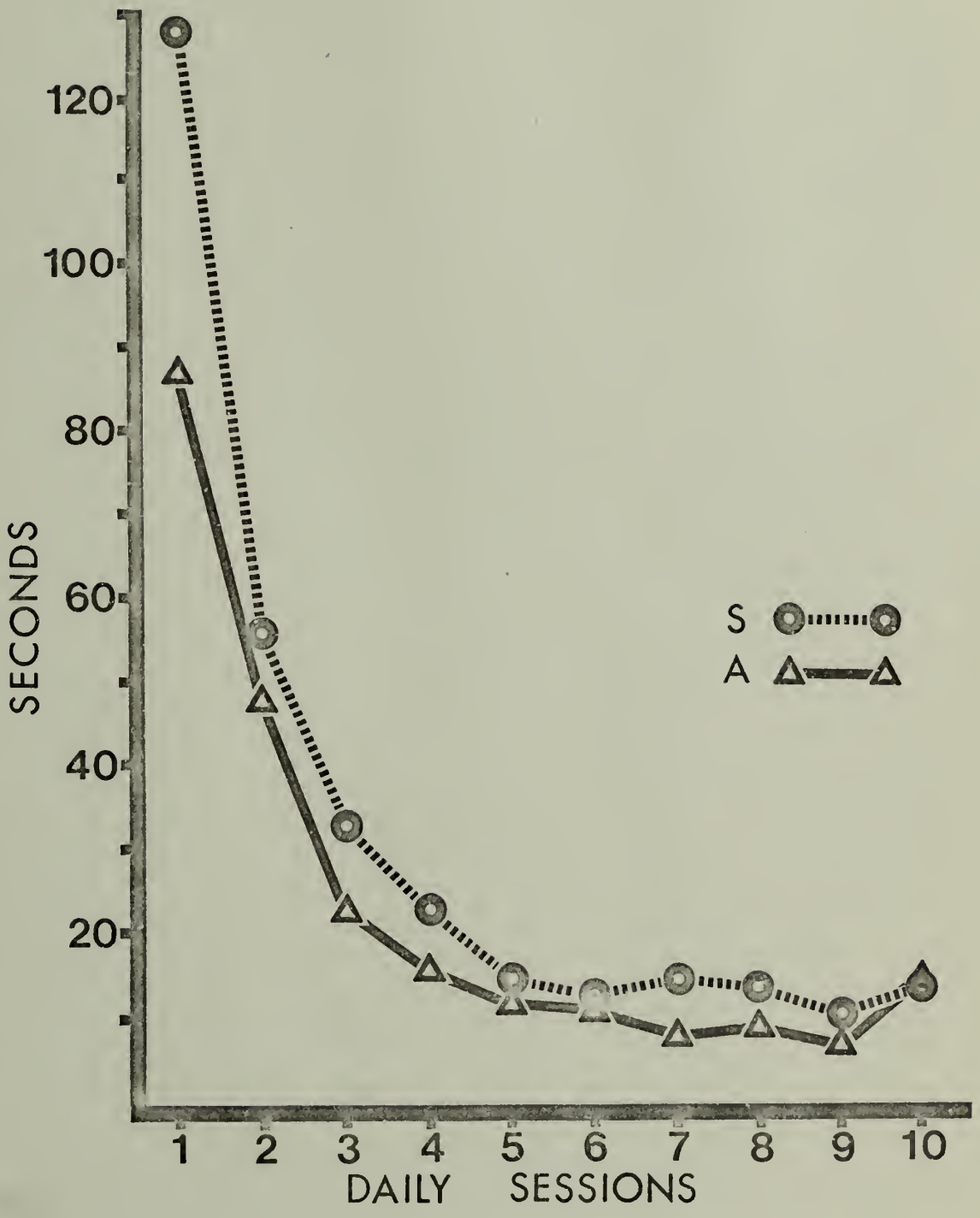
Procedure. The mice were paired on the basis of their DSD behaviors on the last day of pedestal training (prior to maze training). Feeding and pedestal DSD occurred in the same manner as in Experiment 1 except for the stimulation to maintain wakefulness in the Sleepers (and provide matched stress in the Actives). Striking the cage top was generally loud and startling, although its effect on the mice appeared to decrease over the 10 days of training.

Results

Figure 5 shows the performance of the Actives and Sleepers. One Active and one Sleeper had several long latencies to enter the maze and therefore were excluded from the analysis, along with the paired animals. Although Sleepers as a group were consistently slower than Actives in the maze, this difference was small and not significant in an analysis of variance. Neither group differed from the normals of Experiment 2. Again, as in Experiment 4, there was no pronounced difference in score variability between Sleepers and Actives as had been found in Experiments 1 and 3. All analyses showed a significant effect of sessions ($P < .001$), and no significant interactions were detected. Additional analyses indicated that both groups differed from the Sleepers of Experiment 1 (Actives, $F_{1,15} = 12.70$, $P < .003$; Sleepers, $F_{1,15} = 5.21$, $P < .038$), and that neither group differed from Experiment 1 Actives.

It should be added that due to the persistence of sleep posturing in some of the Sleepers, and the high frequency of intense stimulation that was occasionally required, the enforced wakefulness procedure was unquestionably stressful. Indeed it was undoubtedly more so than the DSD procedures previously used in Experiments 1, 3, and 4. Nonetheless, the deficit usually produced in Sleepers was essentially eliminated, and the Actives performed at their typical normal

Figure 5. Summary of Experiment 5. Curves show performance of Actives (A) and Sleepers (S), the latter classification based on screening prior to the enforced wakefulness procedure associated with the post-trial DSD. Actives received matched startling stimulation.



level. Although the primary conclusion from Experiment 5 is that omitting the isolated S sleep eliminates the DSD effect in Sleepers, a secondary point is that the increased level of stress did not result in poorer performance by either group. Therefore, there is strong evidence that the effect of DSD on learning is not due to stress as some critics have suggested.

DISCUSSION

Prior to a fuller consideration of the conclusions and implications drawn from this series of experiments, it is worthwhile to discuss again one of the problems of control in this study. Efforts have been made at several points to demonstrate that prior to DSD, and aside from the effects of isolated S sleep, Sleepers are equivalent to Actives in their learning ability. It has been shown that for the two groups: (1) asymptotic performance is virtually equivalent; (2) likely candidates for the two groups are equivalent in a normal learning situation (without DSD); (3) delay of DSD eliminates the group differences; (4) Sleepers immediately match the performance of Actives (and normals) following the termination of daily DSD at a learning stage when large group differences are present; and (5) enforced wakefulness renders "Sleepers" equivalent to Actives. It was also pointed out that within experiments t tests showed the groups did not differ on Day 1 in the maze, this indicating that the groups could not be differentiated by the amount of initial exploration of the maze. Nonetheless, in 4 of the 5 experiments Sleepers as a group took longer to explore the maze and find the food. Therefore, it is of interest to examine more thoroughly the distributions of Day 1 exploration times for the two groups, with group data collapsed across all experiments. Frequency polygons for these two distributions

are shown in Figure 6. The group means suggest again that Sleepers take longer to find the food (153 sec. vs. 131 sec. for the Actives), but once again the difference proves to be non-significant in a t test ($t_{92} = 1.235$), even with the greater power of the large n (43 Sleepers, 51 Actives).

Both distributions were unimodal and somewhat skewed, with medians lower than the means (Sleepers, 115; Actives, 104). Variances of both groups were high, that of the Sleepers being higher by a factor of 1.56 (S.D. for Sleepers, 96.13; for Actives, 77.03). These variances did not differ in an F test ($F_{42,50} = 1.56$). Thus, evidence is lacking that the groups were constituted in such a way that they differed in ability to learn the maze. Figure 7 shows the distribution of Day 1 exploration times for all animals employed in the study ($n = 94$).

Although there is no basis for asserting that the groups are fundamentally different in their learning ability, it is of course true that these groups must differ in some way. Indeed, the experimental designs employed rest on the clear difference in reactions to the DSD procedure that led to classification of an animal as a Sleeper or as an Active. In such a study an important question to consider is: What determines the reaction of a mouse to the pedestal? In any thorough sense this question remains unanswered. Because the mice used were of uniform genotype, it must be supposed that early experience influences the alignment of some general

Figure 6. Frequency polygons for Day 1 exploration times for Sleepers (S) and Actives (A) collapsed across all experiments. (Classification based on screening in Experiments 2 and 5.)

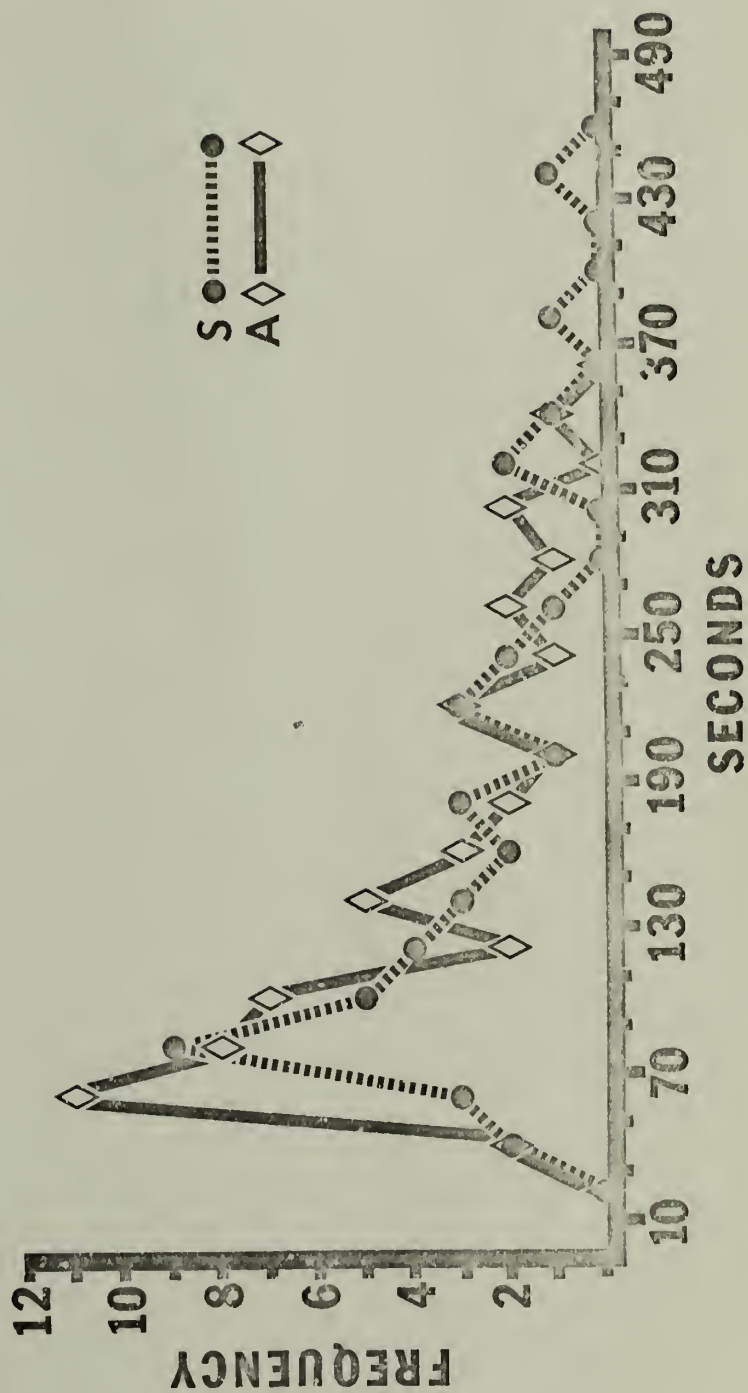
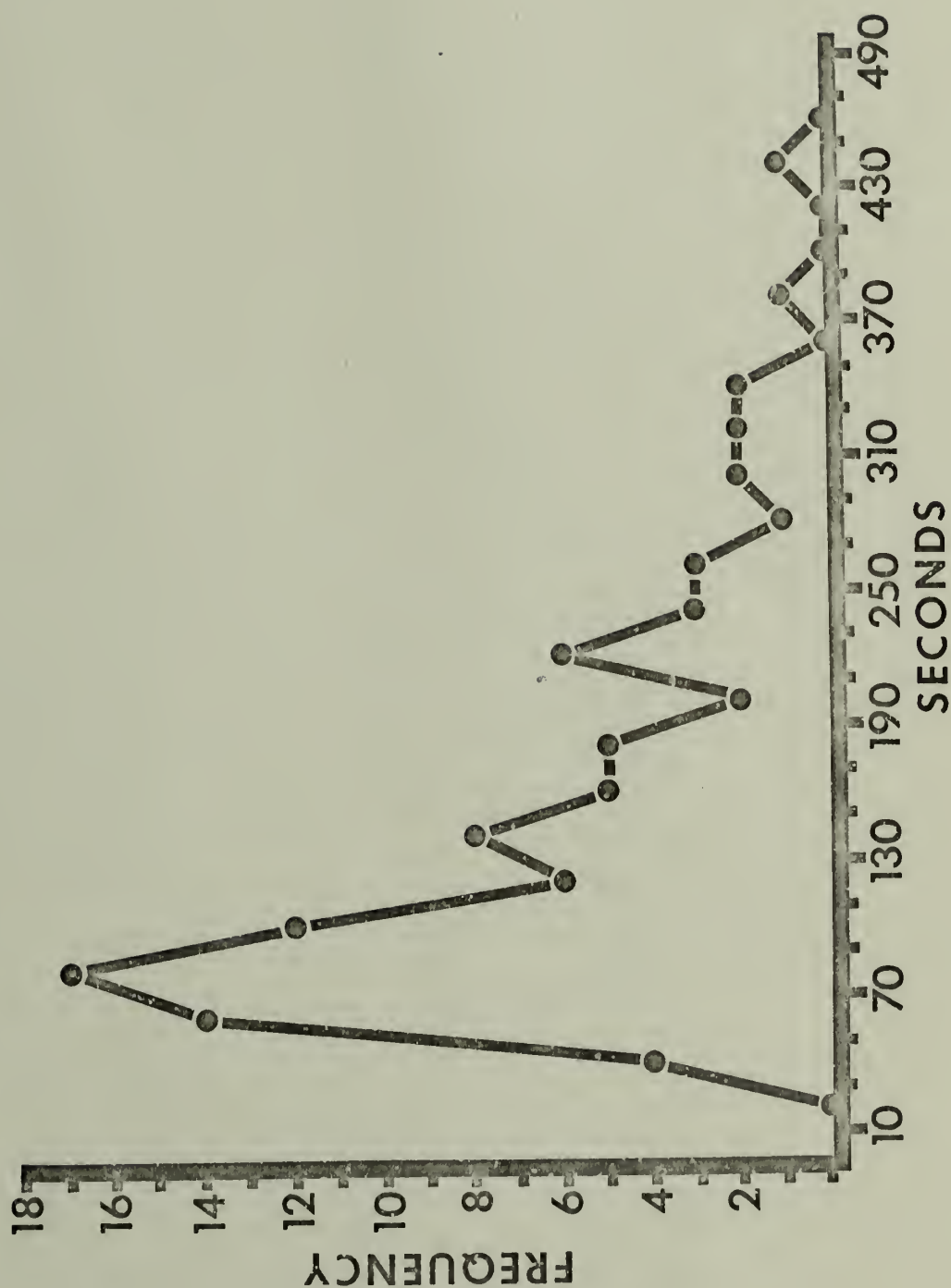


Figure 7. Frequency polygon for Day 1 exploration times for all subjects.



disposition or reaction to stressful circumstances. While the basic determinants of any such predisposition remain unknown, some evidence is available that suggests at least one way in which the reaction can be influenced. In the experiments described above, 40%-60% of the mice generally were found to show a sufficient degree of sleep on the pedestal for classification as Sleepers. These experiments were conducted in the Spring of 1977 and the shipment of mice from the source (about 1 week prior to experimentation) did not involve exposure of the mice to extreme temperatures. In contrast, mice that were shipped and used during the previous winter season showed a much lower proportion of animals classifiable as Sleepers, typically 5%-10%. These latter animals were used in experiments equivalent to Experiments 1 and 2 described above. Data from these earlier experiments have not been included in the present reports, but were consistent with the current findings, despite the much smaller proportion of Sleepers observed. Differences in proportions were not studied in any systematic way, but the parallel between seasonal and reactive change is suggestive. It seems likely that exposure of the very young mice to periods of extreme cold during shipment may have biased their reaction to DSD in favor of remaining active. This interpretation is also consistent with a recent report by Haskell, Walker, Berger, and Heller (1977) that acute exposure to cold causes an increase in

waking time with a concomitant loss in both SS and DS.

With these points clarified we may now consider more fully the results of the present study. The findings of these experiments demonstrate in several ways the validity of the hypothesis in question. Deficits similar to those found in previous DSD experiments have resulted from the pedestal DSD procedure as expected. These deficits, however, have been restricted to those animals showing sleep behaviors during DS deprivation. Animals remaining active during the procedure learned at a normal rate. Thus it seems clear that it is not, strictly speaking, the absence of D sleep that has produced the learning deficit, since all the animals were DS deprived by the procedures. In fact, due to the incompleteness of the DS deprivation in the subjects showing sleep, the Actives rather than the Sleepers were the more thoroughly deprived. Nonetheless, as mentioned above, the Actives performed normally. That the origin of the deficit is in the occurrence of sleep is also indirectly suggested by the differences in within-group variability, which appear for the most part only where the hypothesis predicts that the groups will differ in performance. (Since the use of logarithmically transformed data in these cases either maintains or increases the level of significance in the main effects for grouping, it is clear that the differences in variability are not the cause of the differences in performance between groups.) The higher variability among

Sleepers would be predicted in a general sense by the hypothesized effect of S sleep, since Sleepers may be less homogeneous in the extent of their sleeping than are Actives in their activity. Alternatively, we may note in reference to the Day 1 exploration times that variance in both groups begins high and is much more rapidly reduced by the "treatment" of the Actives, which renders them more homogeneous. Indeed, variance among Actives tends to be slightly lower than among normals of Experiment 2. It should also be pointed out, though, that detectable differences in variance occur primarily during the first 4 days of maze testing.

Although the results of this study require a new perspective on the possible origins of DSD learning deficits, they are not necessarily in conflict with most of the earlier research and may even clarify some of the otherwise contradictory findings. The current results are also generally consistent with a recent trend in the DSD literature to emphasize the importance of heightened CNS activity for information processing and memory consolidation (Bloch, 1976; Fishbein and Gutwein, 1977). This emphasis has naturally been applied to the question of a functional role for D sleep, and the conclusion has been drawn that the absence of the activated brain state of DS is the source of DSD deficits. It is implicit in these (previous) views that DS is in some way especially advantageous to memory consolidation; otherwise, why would not wakefulness serve as well? Although

animals remaining active during DSD were not differentiable from normal control animals in the present study, it is not intended to assert here that D sleep does not on certain occasions serve some special processing function. Application of experimental designs used here to a different task situation might well demonstrate that DSD Actives do not reach the performance level of normal controls. Bloch's (1976) analysis centered around studies that showed some functional similarities (by means of brain activation) between post-trial electrical stimulation of the reticular formation and the naturally occurring activation of D sleep. (Post-trial reticular stimulation prevented the normal DS augmentation associated with learning, and also counteracted the amnesic effect of immediately subsequent fluothane anaesthesia.) From Bloch's perspective the information processing necessary for memory storage encompasses a period starting in wakefulness, during the so-called consolidation phase, and continues during subsequent DS episodes. He suggests that processing during DS involves the "elaboration" of an established trace, rather than its "consolidation." In either case, it is clear that "the important factor" is that these "phases are dependent upon some sort of brain activation," through reticular stimulation or naturally occurring DS.

Fishbein and Gutwein (1977) also attempt an interpretive refinement in an effort to integrate the wide range of DSD

designs and results. These authors also focus on "memory consolidation," and draw a distinction between "conversion" of short-term to long-term memory and "maintenance" of long-term memory. They suggest that these are interfered with by pre- and post-trial DSD, respectively. Interference with "maintenance" would result in a memory trace that is less easily retrievable. These two effects, however, are supposedly mediated by the same changes in transmitter levels in the brain. The extent to which these various distinctions are useful perhaps remains to be seen in future research. A case could be made to some degree for their applicability to the present experiments, since the improvement in performance of Sleepers was so rapid in Experiment 3 (subsequent to termination of daily DSD). It might be said, for example, that (in the Sleepers) the trace is not adequately "elaborated," or that the LTM is not "maintained" in a fashion optimal for retrieval, or that the Actives are substituting the cerebral activation of wakefulness for that of desynchronized sleep. These explanatory comments seem to add little with regard to the present experiments. One may as easily point to a degree of similarity between Pearlman's (1971) demonstration of DSD-induced deficits in the latent learning paradigm and the rapid improvement of Sleepers in Experiment 3. In both cases information may be consolidated, but not optimally intergrated for performing the task.

One may also note here that Fishbein et al. (1977) refer

to a recent failure to replicate (in mice) Pearlman's finding of a "critical period" through use of brief post-trial DSD (in rats). Fishbein et al. found no deficit with the brief DSD procedure, and also noted that this procedure actually "protected" memory from subsequent disruption by ECS. Fishbein et al. conclude that the moderate stress of their procedures enhanced the consolidation, and that in this situation no "critical period" could be demonstrated for mice. Although Fishbein et al. do not mention this point, Fishbein (1976) did mention that the mice in this research were continuously active and thus totally sleep deprived. Thus he seems to imply that the positive effect of stress has overpowered the negative effect of DSD.

As mentioned previously, the primary impact of the present study is to redirect the current implicit perspective on the origin of DSD-induced learning deficits. Although Experiment 5 casts some new light on the question of stress as a factor, the present study has not considered the old issue of whether DSD learning deficits really exist, or instead are artifactual in some fashion. This issue is viewed as non-viable, despite the lingering doubts of occasional authors (e.g. Vogel, 1975). Others (Fishbein and Gutwein, 1977; Greenberg and Pearlman, 1974; Pearlman, 1976) have considered this problem in great detail. Similarly, the current study has nothing to add to the question of what changes in levels

of specific neurotransmitters may result from DSD and may mediate the associated learning deficits. Again, the simple point to be made is only that whatever such changes may be, they most likely have their origin in isolated S sleep, rather than in the mere absence of D sleep during a period of time. It may also be pointed out that in the previous literature much attention has been given to the importance of functional changes in cholinergic and catecholaminergic systems, with impairment in their function and in learning both associated with DSD. In general these systems appear to be active during D sleep. Consistent with the redefining tendency of the present study, it might be appropriate to consider in a complementary fashion that DSD learning deficits may be induced by increases in levels of 5-hydroxytryptamine (5-HT, serotonin). Increases in 5-HT have been demonstrated in various studies mentioned earlier, including particularly the well-controlled study of Cramer et al. (1973). Recently, Kovacevic and Radulovacki (1976) have also shown that 5-HT increases in the hippocampus during S sleep. Specifically germane to the present research, Woolley and van der Hoeven (1963) found that an excess of cerebral 5-HT decreased maze learning ability in mice. Indeed, Essman (1974) has pointed out evidence demonstrating the amnesic effect of 5-HT, and that the common denominator for a number of amnesic agents is the ability to elevate forebrain serotonin. To emphasize increases in 5-HT rather than decreases in other transmitters,

however, may not really involve any dramatic new position, since the systems may well function in opposition to each other (Stein and Wise, 1974), and relative changes may in some cases be equivalent regardless of the terms in which they are stated. In conjunction with the findings concerning 5-HT in the forebrain, it is interesting to note, for example, that hippocampal theta activity, which is characteristic of D sleep and may be involved in memory, appears to be mediated by a cholinergic mechanism (Stumpf, 1965). Furthermore, increases in acetylcholine in the hippocampus can be detected following learning (Matthies, Rauca, and Liebman, 1974), and DSD-induced learning deficits can be reversed with post-trial administration of physostigmine, an anticholinesterase (Skinner, Overstreet, and Orbach, 1976).

Finally, it is appropriate to consider how the results of the present study might relate to the function(s) and evolution of sleep states, or how they might fit within the framework of natural selection. The most immediate implication is probably that D sleep seems to be serving as an antidote to negative effects originating in S sleep. On an information processing basis this point is similar to the neurophysiological perspective of Ephron and Carrington (1966), which invoked homeostatic control of cortical tone as the role of DS. At an ecological level of interpretation, Allison and van Twyver (1970) have also considered that DS may serve to offset a negative effect of S sleep, in this case an increased

susceptibility to danger of predation, with D sleep preparing the CNS for the activated waking state. Thus, the idea of an antidote function of some sort is not really new; but that S sleep per se should be explicitly destructive to information is, at least at a superficial level, not immediately reconcilable with the obvious selective value of memory storage and learning. Nonetheless, this apparent problem can be dealt with in several ways. First, it should be added that this argument is largely moot, since with the exception of the echidna and other monotremes virtually all mammals show both SS and DS. Birds also demonstrate DS and appear to have developed it subsequent to their divergence from the reptiles. But still the question arises why a state would evolve that required another state as antidote. The obvious answer is that S sleep likely bestows advantages that are too great to be given up to wakefulness. The common appearance of SS in birds and mammals, both of which have also developed homeothermy, suggests that the reduced energy consumption of S sleep might be a relevant factor. In fact, in a comparative correlational study of sleep and constitutional variables Zepelin and Rechtschaffen (1974) concluded that S sleep was an enforced state of rest positively correlated with metabolic rate.² Allison

²Note that the use of mice in the current study was perhaps fortuitous on two grounds: first, that this species clearly shows two states during pedestal DSD; and second, that as a species it requires relatively large amounts of S sleep.

and Cicchetti (1976) have extended this type of comparative study to include ecological variables and found similarly that S sleep is negatively associated with a factor related to body size, and also that DS is associated with a factor related to predatory danger. Webb (1974) has also made a case for sleep as a state of adaptive non-responding, whereby animals remain inactive when food is not available, or when danger of predation is high, thus distributing their energy expenditure more efficiently. The restorative aspects of S sleep have also been stressed (Hartmann, 1974).

Some argument can also be made that a degree of information destruction is advantageous, particularly if the most salient experiences from the preceding wakefulness have already been stored in a more or less permanent form, or will be more likely to withstand the effects of S sleep due to greater redundancy of their representation in short-term form. Information erasure or forgetting through sleep is of course not a novel idea either. Gaarder (1966), for example, suggested a computer model for sleep in which one important function of sleep was the "destructuralization of neurophysiological data storage structure." Thus, a deleterious influence of isolated S sleep can be construed as consistent with an evolutionary rationale without any great difficulty.

In summary, it has been demonstrated that a DSD-induced learning deficit, characterized by retarded learning of a

maze, and found to be not the result of stress, occurs only in those animals showing isolated S sleep and can be reversed by brief unrestricted post-trial sleep in the home cage environment. It should be emphasized that viewing D sleep deprivation as the cause of learning deficits is appropriate only if one is describing methods: it appears unjustified to make the logical move from the description of DS deprivation to the assumption that resultant learning deficits are due to the simple absence of D sleep. In a larger sense, as the states of sleep seem to have evolved together, drawing the simplest conclusions from studies of isolated states may be incorrect, regardless of how artfully the isolation may be accomplished.

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